

Hiroshi OKADA\* & Michio TAMURA\*: **Karyomorphological study on the Nymphaeales\*\***

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The order Nymphaeales, consisting of Nymphaeaceae, Nelumbonaceae and Ceratophyllaceae, is one of the most important groups for the systematic and phylogenetic consideration of the primitive angiosperms, and has been studied morphologically and taxonomically to considerable extent. Cytologically this group was investigated by Langlet & Söderberg (1927), Langlet (1936), Ohga et al. (1962), etc.; but the knowledge from this field is still insufficient.

In the present article, the authors report on number and length of metaphase chromosomes and distribution patterns of chromatins at interphase nuclei and prophase chromosomes observed on 12 species belonging to 9 genera of Nymphaeaceae, Nelumbonaceae and Ceratophyllaceae, and discuss their taxonomic and phylogenetic meaning.

**Materials and methods** Sources of plant materials for this study are shown in Tab. 1.

Preparations and observations were done in accordance with the method used in a previous study on the Ranunculaceae (Okada & Tamura 1979). But in *Ceratophyllum* which does not produce roots, juvenile leaves were used. The procedure of preparation for juvenile leaves was described in Okada (1975). It was confirmed on some other species that both methods using root tips and using juvenile leaves showed nearly the same results. Accordingly the features observed in *Ceratophyllum* can be compared with those in other species.

**Observations** Number of chromosomes and length of the largest and the smallest metaphase chromosomes are shown in Tab. 1.

Among the species observed the following 3 types on the chromatic structure of the interphase nuclei and the distribution pattern of hetero- eu- and chromatins at the prophase chromosomes were recognized.

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Tab. 1. Sources, and number and length of chromosomes.

Species	Source	2n	Chr. length max.—min. (μm)
<i>Barclaya mottleyi</i>	Telupid, Sabah, Malaysia	36	3-1.5
<i>Brasenia schreberi</i>	Mt. Hira, Shiga Pref.	80	1-0.5
<i>Cabomba caroliniana</i>	cult. in Kyoto Univ.	ca 96	1.5-0.5
<i>Ceratophyllum demersum</i> var. <i>quadrispinum</i>	Adachi-ku, Tokyo Pref.	72	1-0.5
<i>Euryale ferox</i>	Inami, Hyogo Pref.	58	1-0.5
<i>Nelumbo lutea</i>	cult. in Bot. Gard. Osaka City Univ.	16	3.5-1
<i>Nuphar oguraense</i>	cult. in Bot. Gard. Osaka City Univ.	34	1.5-1
<i>N. subintegerrimum</i>	Sanda, Hyogo Pref.	34	2-1
<i>Nymphaea alba</i>	near Stirling, Scotland, UK.	84	1.5-0.5
<i>N. stellata</i>	near Kota Kinabalu, Sabah, Malaysia	84	1.5-0.5
<i>N. tetragona</i>	Oze, Gumma Pref.	112	2-1
<i>Victoria crusiana</i>	cult. in Bot. Gard. Osaka City Univ.	24	4-2

Fig. 1. Interphase nuclei and somatic chromosomes of Type 1. *Barclaya mottleyi*.A: interphase. B: prophase. C: metaphase.  $\times 1800$ .

Type 1. Species observed: *Barclaya mottleyi*.

Interphase nuclei: Chromatin is observed to be fibrous, and is distributed unevenly in a nucleus and loosely concentrated in 1 to a few regions. The chromatic regions stained rather uniformly.

Prophase chromosomes: Stained almost uniformly and hetero- and euchromatic segments are not distinguishable.

Range of metaphase chromosome length: 1.5-3.5  $\mu\text{m}$ .

Type 2. Species observed: *Victoria crusiana*.

Interphase nuclei: Chromatin is observed to be fibrous and is distributed unevenly in a nucleus as in Type 1. The chromatic regions are not stained uniformly, i.e., parts stained darkly, so-called condensed bodies, and those stained dilutely are distinguished in them. The condensed bodies are several, round-shaped and slightly different from each other in size.

Prophase chromosomes: Hetero- and euchromatic segments are clearly distinguished. Several heterochromatic segments are situated at distal, interstitial and proximal parts in every chromosome arm.

Range of metaphase chromosome length: 2-4  $\mu\text{m}$ .

Type 3. Species observed: *Brasenia schreberi*, *Cabomba caroliniana*, *Ceratophyllum demersum* var. *quadrispinum*, *Euryale ferox*, *Nelumbo lutea*, *Nuphar oguraense*, *N. subintegerrimum*, *Nymphaea alba*, *N. stellata*, *N.*

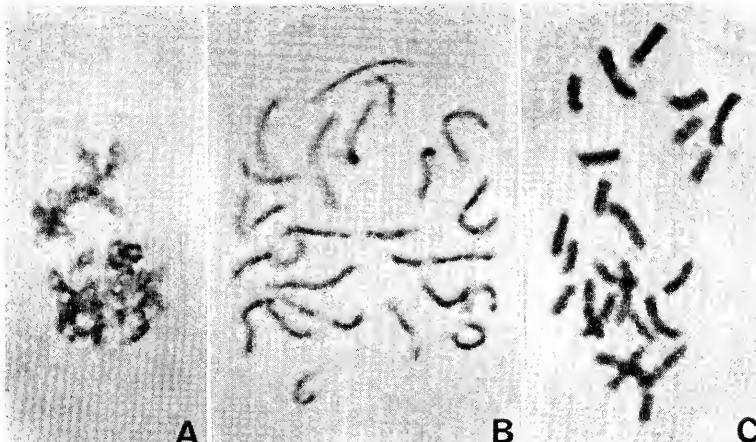


Fig. 2. Interphase nuclei and somatic chromosomes of Type 2, *Victoria crusiana*.

A: interphase. B: prophase. C: metaphase.  $\times 1800$ .

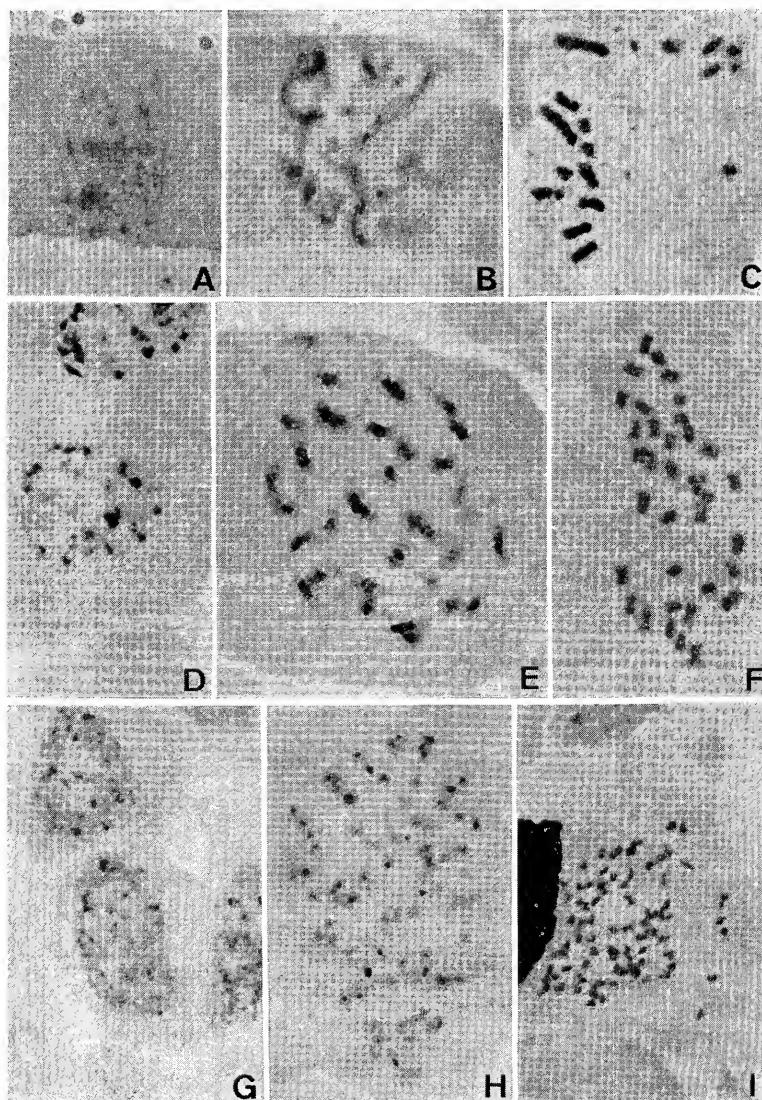


Fig. 3. Interphase nuclei and somatic chromosomes of Type 3. A-C. *Nelumbo lutea*. A, interphase. B, prophase. C, metaphase. D-F. *Nuphar subintegerrimum*. D, interphase. E, prophase. F, metaphase. G-I. *Ceratophyllum demersum* var. *quadrispinum*. G, interphase. H, prophase. I, metaphase.  $\times 1800$ .

*tetragona*.

Interphase nuclei: Chromatin does not show the fibrous appearance and is distributed almost evenly in a nucleus in contrast with the former 2 types.

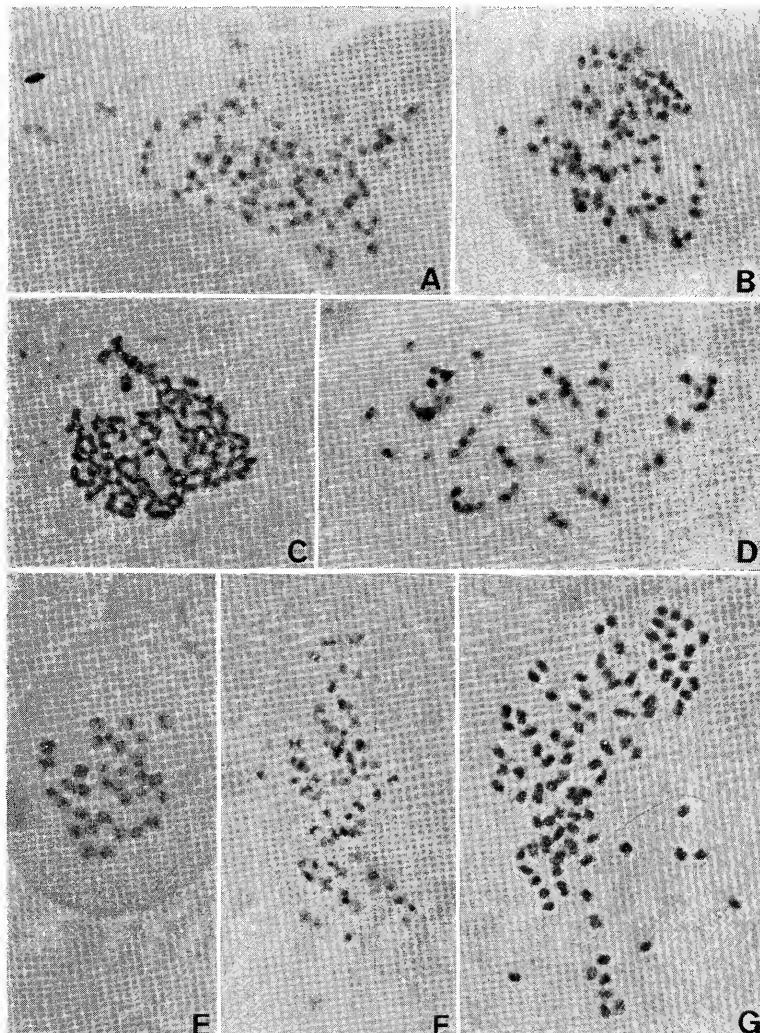


Fig. 4. Somatic metaphase chromosomes of Type 3. A. *Nymphaea alba*. B. *N. stellata*. C. *N. tetragona*. D. *Euryale ferox*. E. *Nuphar oguraense*. F. *Brasenia schreberi*. G. *Cabomba caroliniana*.  $\times 1800$ .

Darkly stained parts, so-called condensed bodies, are distinguished from the dilutely stained base. The condensed bodies are numerous, upto ca 100, and round or rod-shaped, and all of them are nearly equal in size. But in *Nelumbo*, they are only 3 or 4 in number.

Prophase chromosomes: Hetero- and euchromatic segments are clearly distinguished, and one heterochromatic segment is situated at proximal part in every chromosome arm.

Range of metaphase chromosome length: In *Nelumbo* 1-3.5  $\mu\text{m}$ ; in *Nuphar* 1-2  $\mu\text{m}$ ; in others 0.5-1.5  $\mu\text{m}$ .

**Discussion** The chromosome numbers of the nymphaeaceous plants were reported by Langlet & Söderberg (1927), Langlet (1936), Ohga et al. (1962), etc. In the present study, the authors confirmed the diploid numbers  $2n=16$  for *Nelumbo*,  $2n=24$  for *Nuphar*,  $2n=34$  for *Victoria*,  $2n=58$  for *Euryale*, and  $2n=80$  for *Brasenia*. In each of these genera, all the species examined have the same chromosome number, though, except *Brasenia* and *Euryale* which are monotypic, numbers of the species are rather few. The genus *Nuphar* contains fairly many species, but their chromosome numbers are all 34 as far as examined.

In *Nymphaea*, *Cabomba* and *Ceratophyllum*, the chromosome number shows a considerable range of variation and the polyploidal multiplication of chromosome set is recognizable. The results of the present study give the support to the basic numbers  $x=14$  for *Nymphaea*, and  $x=12$  for *Cabomba* and *Ceratophyllum*. In *Nymphaea* the chromosome number ranges from  $2n=28$  ( $2x$ ) for *N. capensis* var. *zanzibariensis* and *N. stellata* (Langlet & Söderberg 1927) upto  $2n=224$  ( $16x$ ) for *N. gigantea* (Langlet 1936).

The fact that the basic chromosome number is 12 in *Cabomba* and *Ceratophyllum* may suggest a certain relationship between both genera. But, as a rule, the numerical relation is obscure among the basic chromosome numbers of the genera of Nymphaeales. It is also remarkable that the family Nymphaeaceae includes both the genus stable in chromosome number such as *Nuphar* and the genus with a wide range of polyploidy such as *Nymphaea*.

On the chromosome number of *Barclaya*, Langlet (1936) reported  $2n=34$  for *Barclaya* sp. and Sokolovska & Melikyan (1964) did  $2n=36$  for *B. longifolia*. The latter number is identical with that of *B. mottleyi* determined in the present study. At the present time it is uncertain whether there is an aneuploidal variation of the chromosome number in the genus or the report of Langlet is

erroneous.

In the Nymphaeales, the chromosomes are small and it is difficult to show the karyotype exactly. Among them, *Victoria crusiana* has the largest chromosome set with chromosomes ranging from 4  $\mu\text{m}$  to 2  $\mu\text{m}$  in length. The chromosome set of *Barclaya mottleyi* with chromosomes ranging from 3  $\mu\text{m}$  to 1.5  $\mu\text{m}$  and that of *Nelumbo lutea* with chromosomes 3.5  $\mu\text{m}$  to 1  $\mu\text{m}$  come next. In all other species examined, the chromosomes are between 2  $\mu\text{m}$  and 0.5  $\mu\text{m}$  in length.

Among the 3 types of karyomorphology found in the Nymphaeales, Type 1 and Type 2 resemble each other in having the interphase nuclei in which chromatin is observed to be fibrous. Such a structure has been found neither in the Ranunculaceae nor in the woody Polycarpicae. Besides, the metaphase chromosomes of *Barclaya mottleyi* and *Victoria crusiana*, representing Type 1 and Type 2 respectively, are comparatively large among the members of the Nymphaeales. These resemblances may show some relation between both genera. *Victoria* resembles *Euryale* in appearance, but both are different from each other in karyomorphology. This fact supports the opinion that *Victoria* and *Euryale* were derived through the different courses of evolution advanced by Miki (1960), Ueno & Kitaguchi (1961), Ueno (1962), etc.

In Type 1, condensed bodies are not observed in the interphase nuclei and the prophase chromosomes are stained rather uniformly, while in Type 2 and Type 3 condensed bodies are observed in the interphase nuclei and hetero- and euchromatic segments are distinguished in the prophase chromosomes.

Among the genera with Type 3, *Nelumbo* is slightly different from others in having less (3 or 4) condensed bodies in the interphase nuclei and comparatively large (1-3.5  $\mu\text{m}$ ) metaphase chromosomes. Accordingly, it may be better to distinguish the karyomorphology of *Nelumbo* from others as a subtype of Type 3.

Type 3 resembles 3rd type of the Ranunculaceae and C type of the woody Polycarpicae. But this type is considered to be rather specialized in combining with small chromosomes, and genera which show this type of karyomorphology are classified in various taxonomic groups.

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スイレン目 (スイレン科・ハス科・マツモ科を含む) に属する 9 属 12 種の根端細胞について, 前期・中期染色体, および中間期核を観察した。マツモは根を生じないので若い葉を代りに用いた。いくつかの種について根端と幼葉の細胞を比較したが, 両方で差はほとんど認められず, 両者を比較する上で支障のないことを確認した。

中期染色体の数と長さは表 1 に示されている。数については従来の報告を確認する結果が得られた。ただ *Barclaya* については  $2n=34$  と  $2n=36$  という報告があるが, この研究の結果は後者と一致する。前者が間違っているのか, またはこの属の染色体数に異数的变化があるのかは今のところ不明である。この群の染色体は小形であり, 核型を正確に決定するのは困難である。これらのうちでは *Victoria* のものがもっとも大きく, *Barclaya* とハス属のものがそれに次いでいる。

中間期核と前期染色体における染色質の凝縮状態について観察し, 次の 3 型を区別した。

第 1 型, 中間期核において染色質は糸状に見え, 核内でいくつかの部分に集まるが, その中では均質に染まる。前期染色体で異質染色質と真正染色質とは区別できない。*Barclaya* で見られる。

第 2 型, 中間期核において染色質は糸状に見え, いくつかの部分に集ることは第 1 型

と同様であるが、その中にいくつかの濃く染まる凝縮塊が認められる。前期染色体では異質染色質の部分と真正染色質の部分は明らかに区別される。*Victoria* で見られる。

第3型、前の二つの型とは異なり、中期核で染色質は糸状に見えず、核全体に拡がる。その中に多数の凝縮塊が認められる。前期染色体では異質染色質の部分と真正染色質の部分は明らかに区別される。ジュンサイ属、ハゴロモモ属、コウホネ属、スイレン属、オニバス属、マツモ属、ハス属。これらのうちハス属では、中期核にみられる凝縮塊が3、4個しかなく、染色体も大きく、他の属との違いが認められる。

これら3型のうち、第1型と第2型は中期核において染色質が糸状に見えて集りをつくることで類似しており、第2型と第3型は中期核に凝縮塊が見られること、前期染色体に異質染色質と真正染色質の部分が区別されることで類似している。

オニバス属と *Victoria* は外見はよく似ているが染色質凝縮状態が異なっており、このことは、主として種子の表皮細胞や花粉の特徴にもとづいて唱えられた、両属は別な由来をもつという三木、上野らの意見を支持する。

第3型はキンポウゲ科や木本性多心皮類にも見られ、染色体の小形化とむすびついた特殊化と思われ、この型をもつ属は必ずしも系統的に連関づけられるとはかぎらない。

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□Berggren, Greta: *Atlas of seeds* Part 3. 259 pp. 1981. Swedish Museum of Natural History. 種子の図鑑である。表記の題目に統いて and small fruits of Northwest-European plant species (Sweden, Norway, Denmark, East Fennoscandia and Iceland) with morphological descriptions. Salicaceae—Cruciferae とあるように北欧地域の植物の種子および小果実を分類順に記相し、写真をつけたものである。科および属の見出しの下に全体の種子の記述と属、種の key があり、種ごとにくわしい形態学的記述が続く。図版は 105 あり、種子の各方向からの写真、断面、変異幅、図などが並んでいる。ところどころに走査電顕像もある。写真は輪画や稜線に墨が入っていて、時に強調されすぎている感じをうけるものがある。種子断面では Hypocotyl の位置に特異性のあるものがあり、興味をそそられる。わが国の植物については断片的な種子図鑑があるが、こういう工合にまとまったものはない。どうもこののような枚挙記載的なものは分類学者の間でさえソッポを向かれる傾向があつて残念だが、研究上も実用上も大変有用なものなので、続刊を期待すると共にわが国でも手をつける人が出ることを希望する。

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